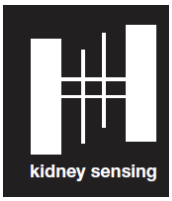
# Preparation of B. subtilis competent cell

## Materials

- Sterile 1.5 mL microtubes
- LB broth
- T base – Autoclave
  - $(NH_4)SO_4$ 2g
  - $K_2HPO_4.3H_20$ 18.3g
  - $KH_2PO_4$ 6g
  - Trisodium citrate . $2H_20$ 1g
- SpC – Made fresh on day
  - T base 20ml
  - 50%(w/w) glucose 0.2ml
  - 1.2%(w/w) $MgSO_4.3H_20$ 0.3ml
  - 10%(w/w) Bacto yeast extract 0.4ml
  - 1%(w/w) tryptone 0.5ml
- SpII – Made fresh on day
  - T base 200ml
  - 50%(w /w) glucose 2ml
  - 1.2%(w/w) $MgSO_4.3H_2O$ 14ml
  - 10% Bacto yeast extract 2ml
  - 1%(w/v) tryptone 2ml
  - 0.1 M $CaCl_2$ 1ml

## Apparatus

- Centrifugue
- Spectrofotometer
- Shaker
- Flow chamber
- Autoclave




## Method

1. Streak out the strain to be made competent on an LB agar plate as a large patch and incubate overnight at 30°C;
2. The following morning scrape the cell growth off the plate and use to inoculate fresh, pre-warmed, SpC medium (20 mL) to give an $OD_{600}$ reading of about 0.5;
3. Incubate the culture at 37 ºC with vigorous aeration and take periodic OD readings ($OD_{600}$) to assess cell growth;
4. When the rate of cell growth is seen to depart from exponential (i.e. no significant change in cell density over 20-30 min) inoculate 200 mL of pre-warmed, SpII medium with 2 mL of stationary-phase culture and continue incubation at 37 ºC with slower aeration;
5. After 90 min incubation, pellet the cells by centrifugation (4,000 g, 25min) at room temperature;
6. Carefully decant the supernatant into a sterile container and save;
7. Gently resuspended the cell pellet in 2,25 mL of the saved supernatant, mix gently.